

### AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

#### LISTING OF CLAIMS

1. (Withdrawn) A method of detecting and quantifying a cell type of interest among various types of cells, said method comprising the steps of:

- providing a test sample having target cells, said target cells having a common surface marker, said target cells including a first cell type having a first surface marker and a second cell type having a second surface marker;

- providing a bio-disc, said bio-disc comprising a substantially circular substrate having a center and an outer edge, a target zone disposed between said center and said outer edge, a plurality of capture antibodies bound to said substantially circular substrate within said target zone such that said plurality of capture antibodies are immobilized in said target zone, said plurality of capture antibodies having affinity for said common surface marker on said target cells;

- depositing said test sample on said target zone;

- allowing said target cells including said first and second type cells present in said test sample to bind with said plurality of capture antibodies through said common surface marker;

- washing said target zone to remove any unbound cells;

- providing a reporter agent having a signal antibody bound thereto, said signal antibody having specific affinity to said first surface marker;

- depositing said reporter agent on said target zone;

- allowing said signal antibody to bind to said first surface marker on said first cell type in said target zone to thereby tag said first cell type;

- washing the target zone to remove any unbound reporter agent; and

- scanning a beam of electromagnetic radiation over said target zone to thereby determine the number of tagged first cell type cells and untagged second cell type cells.

2. (Withdrawn) The method of claim 1 wherein said reporter agent is a bead having a detectable physical property.

3. (Withdrawn) The method of claim 2 wherein said detectable physical property is selected from the group consisting of color, size, texture, reflectivity, absorbance, mass, fluorescence, phosphorescence, and magnetic properties.

4. (Withdrawn) The method of claim 1 wherein said reporter agent is an enzyme.

5. (Withdrawn) The method of claim 4 further comprising the step of depositing onto said target zone an enzyme substrate that reacts with said enzyme to produce a detectable signal.

6. (Withdrawn) The method of claim 5 wherein said enzyme is selected from the group consisting of horseradish peroxidase and alkaline phosphatase.

7. (Withdrawn) The method of claim 6 wherein said enzyme substrate is selected from the group consisting of TMB (3,3', 5,5'-tetramethyl benzidine), DAB (3,3'-Diaminobenzidine), ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)), AEC (3-Amino-9-ethylcarbazol), NBT (Nitro Blue Tetrazolium), CN/DAB (4-chloronaphthol/3,3'-diaminobenzidine, tetrahydrochloride), and 4-CN (4-chloro-1-naphthol).

8. (Withdrawn) The method of claim 7 wherein said detectable signal is a precipitate, which adheres to said first cell type.

9-13. (Canceled)

14. (Withdrawn) A method of detecting and quantifying a cell type in a test sample having various types of cells, said method comprising the steps of:

providing a bio-disc including a substantially circular substrate having a center and an outer edge, a target zone disposed between the center and the outer edge, a plurality of capture antibodies bound to said substrate within said target zone such that said plurality of capture antibodies are immobilized in said target zone, said plurality of capture antibodies having affinity for a common antigen among said various types of cells;

depositing said test sample on said target zone;

allowing said plurality of capture antibodies to bind with said common antigen to immobilize said various types of cells in said target zone;

washing said target zone to remove unbound cells;

providing at least one group of signal antibodies, each antibody thereof having attached thereto a reporter agent unique for said group, each group of signal antibodies having affinity to one cell type among the various types of cells immobilized in said target zone;

allowing said at least one group of signal antibodies to bind to its respective cell type to thereby tag said respective cell type;

washing said target zone to remove unbound signal antibodies; and

scanning a beam of electromagnetic radiation over said target zone to thereby determine the number of untagged cells and cells tagged with said at least one group of signal antibodies.

15. (Original) An optical bio-disc for detecting a cell type among various cell types in a sample, said optical bio-disc comprising:

a substantially circular substrate having a center and an outer edge;

an active layer associated with said substrate;

a target zone disposed between said center and said outer edge; and

a plurality of capture antibodies bound to said active layer such that said antibodies are immobilized on said active layer in said target zone.

16. (Original) The optical bio-disc according to claim 15 wherein said active layer is selected from the group consisting of nitrocellulose, polystyrene, polycarbonate, polystyrene-co-maleic anhydride, and gold.

17. (Original) The optical bio-disc according to claim 16 wherein said substrate includes encoded information associated therewith, said encoded information being readable by a disc drive assembly to control rotation of said bio-disc.

18. (Original) The optical bio-disc according to claim 17 further comprising a reflective layer formed on a surface of said substrate.

19. (Original) The optical bio-disc according to claim 18 further comprising a flow channel in fluid communication with said target zone and an input site in fluid communication with said flow channel.

20. (Original) The optical bio-disc according to claim 19 further comprising an enzyme, wherein said enzyme, when exposed to an enzyme substrate, produces a signal detectable by an incident beam of electromagnetic radiation.

21. (Original) The optical bio-disc according to claim 19 wherein said plurality of capture antibodies have affinity to a common surface marker on cells.

22. (Withdrawn) A method of using the optical bio-disc of claim 21 for differentiating and quantifying a cell type of interest, said method comprising the steps of:

- providing a sample containing said cell type of interest and a non-target cell type, both cell types having said common surface marker, said non-target cell type having a unique surface marker;

- providing a tagging agent attached to an antibody having affinity to said unique surface marker on said non-target cell type;

- mixing said tagging agent with said sample;

- allowing said tagging agent to bind with said unique surface marker on said non-target cell type to thereby block said common surface marker on said non-target cell type thus preventing binding of said non-target cell type to said plurality of capture antibodies in said target zone;

- loading said sample into said flow channel through said input site to place said sample in contact with said capture antibody in said target zone;

- allowing said capture antibody to bind with said common surface marker on said cell type of interest;

- rotating said optical bio-disc at a pre-determined speed and duration to remove unbound non-target cells from the target zone; and

- scanning a beam of electromagnetic radiation over said target zone to determine the number of said cell type of interest in said target zone.

23. (Withdrawn) The method according to claim 22 wherein said sample are mononuclear cells including lymphocytes and monocytes.

24. (Withdrawn) The method according to claim 23 wherein said common surface marker is a CD4 antigen.

25. (Withdrawn) The method according to claim 24 wherein said unique surface marker is a CD14 antigen.

26. (Withdrawn) A method of identifying and quantifying various cell types using the optical bio-disc ~~made according to claim 12~~ according to claim 15, said method comprising the steps of:

- providing a sample containing said various cell types having said common surface antigen, said various cell types including a first cell type having a first surface marker, a second cell type having a second surface marker and a third cell type having a third surface marker;

- loading said sample into said flow channel through said inlet port to place said sample in contact with said plurality of capture antibodies within said target zone;

- allowing said plurality of capture antibodies to bind with said common surface antigen to immobilize said various cell types in said target zone;

- rotating said optical bio-disc at a pre-determined speed and time to remove unbound cells from the target zone;

- providing a labeling solution containing different types of reporter agents, said different types of reporter agents including a first reporter agent having attached thereto a first signal antibody with affinity to said first surface marker, a second reporter agent having attached thereto a second signal antibody with affinity to said second surface marker and a third reporter agent having attached thereto a third signal antibody with affinity to said third surface marker;

- loading said labeling solution into said flow channel through said inlet port to place said reporter agents in contact with said cells immobilized in said target zone;

- allowing said first signal antibody to bind with said first surface marker on said first cell type in said target zone thereby labeling said first cell type with said first reporter agent;

- allowing said second signal antibody to bind with said second surface marker on said second cell type in said target zone thereby labeling said second cell type with said second reporter agent;

allowing said third signal antibody to bind with said third surface marker on said third cell type in said target zone thereby labeling said third cell type with said third reporter agent;

rotating said optical bio-disc at a pre-determined speed and time to remove unbound reporter agents from the target zone; and

scanning a beam of electromagnetic radiation over said target zone to thereby determine the number of cells tagged with said first reporter agent, the number of cells tagged with said second reporter agent and the number of cells tagged with said third reporter agent.

27. (Withdrawn) The method according to claim 26 wherein said sample are mononuclear cells.

28. (Withdrawn) The method according to claim 27 wherein said common surface antigen is a CD45 antigen.

29. (Withdrawn) The method according to claim 28 wherein said plurality of capture antibodies are anti-CD45 antibodies.

30. (Withdrawn) The method according to claim 29 wherein said first cell type is a lymphocyte.

31. (Withdrawn) The method of claim 30 wherein said first surface marker on said first cell type is selected from the group consisting of CD3, CD19 and CD56 antigens.

32. (Withdrawn) The method of claim 31 wherein said first signal antibody is selected from the group consisting of anti-CD3 antibody, anti-CD19 antibody, and anti-CD56 antibody.

33. (Withdrawn) The method of claim 29 wherein said second cell type is a monocyte.

34. (Withdrawn) The method of claim 33 wherein said second surface marker on said second cell type is a CD14 antigen.

35. (Withdrawn) The method of claim 34 wherein said second signal antibody is an anti-CD14 antibody.

36. (Withdrawn) The method of claim 29 wherein said third cell type is a eosinophil.

37. (Withdrawn) The method of claim 36 wherein said third surface marker on said third cell type is a CD116 antigen.

38. (Withdrawn) The method of claim 37 wherein said third signal antibody is an anti-CD116 antibody.

39. (Withdrawn) A method of detecting and quantitating target cells in a test sample, said method comprising the steps of:

- providing a bio-disc, said bio-disc comprising a substantially circular substrate having a center and an outer edge, an active layer associated with said substrate, a target zone disposed between said center and said outer edge, at least one capture antibody bound to said active layer such that said capture antibody is immobilized on said active layer in said target zone, said capture antibody having affinity to said target cell;

- depositing said test sample on said target zone;

- allowing any target cell present in said test sample to bind with said capture antibody;

- washing said target zone to remove any unbound cells;

- providing a plurality of signal antibodies, each signal antibody having an affinity agent that binds to a binding agent;

- allowing said signal antibodies to bind to any target cell that have antigens recognized by said signal antibodies to thereby tag any target cell;

- washing said target zone to remove any unbound signal antibodies;

- providing a plurality of reporter agents, each reporter agent having one or more binding agents;

- allowing said reporter agents to bind to any signal antibody bound to any target cell in said target zone;

- washing said target zone to remove any unbound reporter agents; and

- scanning a beam of electromagnetic radiation over said target zone and analysing a return beam to thereby determine the number of tagged and untagged cells.

40. (Withdrawn) The method of claim 39 wherein said reporter agent is a bead having a physical property detectable using an optical disc reader.

41. (Withdrawn) The method of claim 40 wherein said physical property is selected from the group consisting of color, size, texture, reflectivity, absorbance, mass, fluorescence, phosphorescence, and magnetic properties.

42. (Withdrawn) The method of claim 39 wherein said reporter agent is an enzyme.

43. (Withdrawn) The method of claim 42 further comprising the step of depositing onto said target zone at least one enzyme substrate that reacts with said enzyme to produce a detectable signal.

44. (Withdrawn) The method of claim 39 wherein said affinity agent is biotin.

45. (Withdrawn) The method of claim 42 wherein said enzyme is conjugated with a binding agent that binds to said affinity agent.

46. (Withdrawn) The method of claim 45 wherein said binding agent is selected from the group consisting of streptavidin and neutravidin.

47. (Withdrawn) The method of claim 42 wherein said enzyme is selected from the group consisting of horseradish peroxidase and alkaline phosphatase.

48. (Withdrawn) The method of claim 43 wherein said enzyme substrate is selected from the group consisting of TMB (3,3', 5,5'-tetramethyl benzidine), DAB (3,3'-Diaminobenzidine), ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)), AEC (3-Amino-9-ethylcarbazol), NBT (Nitro Blue Tetrazolium), CN/DAB (4-chloronaphthol/3,3'-diaminobenzidine, tetrahydrochloride), and 4-CN (4-chloro-1-naphthol).

49. (Withdrawn) The method of claim 48 wherein said detectable signal is a precipitate, which adheres to the target cell.

50. (Withdrawn) The method of claim 39 wherein said target cell is selected from the group comprising white blood cells, tumor cells, bacteria, virus, unicellular organisms, cell from tissues and cells from organs.

51. (Withdrawn) A method of detecting and quantifying a target cell, said method comprising the steps of:

providing a bio-disc including a substantially circular substrate having a center and an outer edge, a target zone disposed between the center and the outer edge, an affinity agent bound to said substrate within said target zone such that said affinity agent is immobilized in said target zone;



providing a sample containing said target cell, said target cell having a surface marker;

mixing a reporter agent with said sample, said reporter agent having attached thereto a capture antibody and a binding agent, said capture antibody

having affinity to said surface marker, said binding agent having affinity to said affinity agent;

allowing said capture antibody to bind with said surface marker on said target cell to thereby tag said target cell with said reporter agent,

depositing said sample with said tagged target cell on said target zone; allowing said binding agent attached to said reporter agent to bind with said affinity agent to immobilize said target cell in said target zone;

washing said target zone to remove unbound cells; and

scanning a beam of electromagnetic radiation over said target zone to thereby determine the number of cells tagged with said reporter agent.

52. (Withdrawn) The method of claim 51 wherein said target cell is selected from the group comprising white blood cells, tumor cells, bacteria, virus, unicellular organisms, cell from tissues and cells from organs.

53. (Withdrawn) The method of claim 52 wherein said affinity agent is biotin.

54. (Withdrawn) The method of claim 52 wherein said binding agent is selected from the group consisting of streptavidin and neutravidin.

55. (New) The optical bio-disc according to claim 15 wherein said plurality of capture antibodies comprises populations of antibodies, each population having an affinity to a different cell surface marker.

56. (New) The optical bio-disc according to claim 55 further comprising cells bound to antibodies from at least one population of antibodies.

57. (New) The optical bio-disc according to claim 56 wherein said cells comprise blood cells.

58. (New) The optical bio-disc according to claim 57 wherein said blood cells comprise lymphocytes.

59. (New) The optical bio-disc according to claim 56 wherein at least some of said cells are attached to one or more beads.

60. (New) The optical bio-disc according to claim 56 wherein all of said cells are attached to one or more beads.

61. (New) The optical bio-disc according to claim 15 wherein said plurality of capture antibodies comprises a single population of antibodies, said population having an affinity to a single cell surface marker.

62. (New) The optical bio-disc according to claim 61 further comprising cells bound to said capture antibodies.

63. (New) The optical bio-disc according to claim 62 wherein said cells comprise blood cells.

64. (New) The optical bio-disc according to claim 63 wherein said blood cells comprise lymphocytes.

65. (New) The optical bio-disc according to claim 62 wherein at least some of said cells are attached to one or more beads.

66. (New) The optical bio-disc according to claim 62 wherein all of said cells are attached to one or more beads..